EXPERIMENTAL STUDY

Effects of fullerenol nanoparticles on kidney tissue in sevoflurane-treated rats

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ABSTRACT

AIM: The aim of this study is to demonstrate whether fullerenol C60 protects renal injury in sevoflurane-administered rats.

METHOD: Rats (n: 24) were randomly divided into four groups: Control (Group C), Fullerenol C60 (Group F), Sevoflurane (Group S), Fullerenol C60-Sevoflurane (Group FS). Thirty minutes before the procedure, Fullerenol C60, 100 mg/kg, was administered intraperitoneally. Sevoflurane (2.3 %) was applied for 3 hours to rats in S and FS groups. Biochemical and histopathological parameters were analyzed in renal tissue samples. Kruskal–Wallis and Mann–Whitney U tests were used in statistical analyzes.

RESULTS: Malondialdehyde (MDA) level and catalase (CAT) enzyme activity in Group S were significantly higher than that in all other groups. Paraoxanase (PON) enzyme activity in Group S was significantly lower than in Groups C and FS. The histopathological examination showed that vascular vacuolization and hypertrophy (VVH) and lymphocyte infiltration (LI) were significantly higher in the Group S compared to the Group C.

CONCLUSION: Renal histopathology revealed that the administration of Fullerenol C60 prior to sevoflurane inhalation reduced oxidative stress and partially corrected the damage caused by anesthesia. We concluded that Fullerenol C60 has a renal protective effect in rats when administered before sevoflurane anesthesia (Tab. 2, Fig. 4, Ref. 40). Text in PDF www.elis.sk.

KEY WORDS: fullerenol C60, sevoflurane, renal injury, MDA, PON-1.

Introduction

Fullerenol C60 (OH) is a water-soluble analog of fullerene and forms polyanion nanoparticles (1). In vivo and in vitro studies demonstrated that polyhydroxylated fullerenes had high antioxidative activity (2–4). It was shown that the fullerene-related antioxidant action depends on reorganization of double π bonds on the surface of the molecule. Beside this high antioxidant effects, studies reported that fullerenes demonstrate radical scavenging activity, anti-genotoxic effect and protection against drug-induced toxicity (2–9).

Sevoflurane, the most preferred inhaled agent in anesthesia maintenance, is a halogenated anesthetic, and has favorable physiochemical and pharmaco-dynamic properties. Its low blood solubility facilitates rapid induction and recovery from anesthesia, thus providing a better control of anesthetic depth during the maintenance when compared to other commonly used volatile agents (10, 11). In both animals and humans, the biotransformation of sevoflurane by the hepatic microsomal (cytochrome P450) enzyme system results in generating inorganic fluoride ions, which in turn are capable of producing hepatic and especially renal toxicity (12, 13). Moreover, upon contact with alkaline CO2 absorbents (particularly those containing potassium hydroxide), sevoflurane is degraded to compound A (fluoromethyl-2,2-difluoro-1-((trifluoromethy) vinyl ether) and when inhaled, it is nephrotoxic in rats (14–16). It also has been associated with changes in biochemical markers of renal injury in humans (17–20). There are numerous studies aimed at examining sevoflurane’s hepato- and nephrotoxic potential in humans (13, 17–30) and several laboratory animal species (15, 16, 31, 32) but there have been no studies focused on investigating the influence of fullerenols on sevoflurane anesthesia-related renal injury.

In the present study we aimed to investigate the effects of fullerenols on sevoflurane associated renal injury in a rat model.

Material and methods

The present study was carried out in the Experimental Animals Laboratory of the Gazi University Medical Faculty (Ankara, Turkey), and was approved by the Gazi University Ethics Committee of Experimental Animals. All methods were in accordance with...
the Guide for the Care and Use of Laboratory Animals (Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health, NIH Publication no. 85–23, revised, 1996).

Twenty-four male Wistar rats weighing 250–330 grams were used in this study. Rats were kept in separate cages, at a 12-hour light and dark cycle at room temperature (24 °C). They had free access to standard rat chow and water. The rats were randomly divided into four groups: Control (Group C), Fullerol C60 (Group F), Sevoflurane (Group S), Fullerol C60-Sevoflurane (Group FS). Before inducing the anesthesia, namely 30 minutes before the procedure, 100 mg/kg intraperitoneal nanoparticle was administered and sevoflurane 2.3 % was applied to rats in S and FS groups for 3 hours. Renal tissue samples were taken at the end of the anesthesia period.

**Processing and preparation of tissue**

The tissue specimens were rapidly excised, washed in ice-cold normal saline, blotted, frozen in liquid nitrogen, and stored at −80 °C until use. 10 % (w/v) homogenization of kidney tissues was done in Tris- HCl (0.1 M, pH 7.4) using an ice-chilled glass homogenizing vessel in a homogenizer fitted at 15,000 rpm. The suspended mixture was centrifuged at 1,000 g for 10 min at 4 °C in a refrigerated centrifuge.

**Kidney malondialdehyde (MDA) level assay**

The extent of lipid peroxidation was determined as the concentration of thiorbituric acid reactive substances (TBARS) according to the method of Ohkawa et al (33). Briefly, 100 μL of kidney homogenates or MDA standards were pipetted into test tubes containing 1.5 mL of 20 % (w/v) glacial acetic acid (pH 3.5), 200 μL of 8.1 % (w/v) sodium dodecyl sulphate (SDS), 1.5 mL of 0.8 % (w/v) thiorbituric acid (TBA) and 250 μL of distilled water. The test tubes were incubated at 95 °C for 60 minutes with a marble on top of each test tube. After incubation, the test tubes were cooled and then centrifuged at 4,000 × g for 10 minutes. The amount of MDA formed was measured spectrophotometrically at 532 nm. 1,1,3,3-Tetraethoxypropane (TEP), a form of MDA, was used as standard in this assay. TBARS concentration was expressed as nmol of MDA per mg protein.

**Kidney catalase (CAT) enzyme activity assay**

A calorimetrically enzymatic assay kit at 405 nm (ZellBio GmbH, Ulm, Germany) was used to measure CAT activity. The amount of the sample that contributes to decomposition of 1 μmol of H2O2 to water and O2 in one minute is considered as one CAT activity unit. The sensitivity of this assay is about 0.5 U/mL.

**Paraoxanase (PON) enzyme activities**

PON enzyme activity was determined spectrophotometrically at 25 °C using diethyl p-nitrophenyl phosphate (paraoxon; 1 mM) in 50 mM glycine/NaOH (pH 10.5) containing 1 mM CaCl2. The enzyme assay was based on the estimation of p-nitrophenol at 412 nm. The molar extinction coefficient of p-nitrophenol (ε = 18,290 M −1 cm−1 at pH 10.5) was used to calculate the enzyme activity. One enzyme unit was defined as the amount of enzyme that catalyzed the hydrolysis of 1 μmol of substrate at 25 °C.

**Histological determinations**

All of the specimens were fixed in 10 % buffered neutral formalin and embedded in paraffin. To visualize myocardial lesions at different levels, the entire heart was cut into four segments from apex to bottom. The segments were embedded in paraffin and 4-μm thickness cross-sections were cut from each segment. The slides were stained with Hematoxylin-Eosin (Bio-optica, Milano, Italy) for the evaluation of the tissues’ histological features.

**Statistical analysis**

SPSS version 20.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The differences between groups were as-

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**Table 1. Data regarding oxidative status of renal tissue (mean ± SD).**

<table>
<thead>
<tr>
<th></th>
<th>Group C (n=6)</th>
<th>Group F (n=6)</th>
<th>Group S (n=6)</th>
<th>Group FS (n=6)</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>1.21±0.14*</td>
<td>1.07±0.15*</td>
<td>2.31±0.65</td>
<td>0.97±0.17*</td>
<td>0.048</td>
</tr>
<tr>
<td>CAT (IU/mg protein)</td>
<td>1463.60±144.88*</td>
<td>1687.40±120.24*</td>
<td>2124.25±152.99</td>
<td>1416.60±98.58*</td>
<td>0.008</td>
</tr>
<tr>
<td>PON (IU/mg protein)</td>
<td>723.83±61.03*</td>
<td>655.00±35.05</td>
<td>520.50±51.65</td>
<td>671.00±40.95*</td>
<td>0.045</td>
</tr>
</tbody>
</table>

* - Kruskal–Wallis p < 0.05; **p < 0.05: Compared to Group S

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**Table 2. Histopathological data of renal tissue (mean ± SD).**

<table>
<thead>
<tr>
<th></th>
<th>Group C (n=6)</th>
<th>Group F (n=6)</th>
<th>Group S (n=6)</th>
<th>Group FS (n=6)</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glomerular vacuolization (GV)</td>
<td>0.33±0.21</td>
<td>0.67±0.21</td>
<td>0.83±0.31</td>
<td>0.67±0.21</td>
<td>0.523</td>
</tr>
<tr>
<td>Tubular dilation (TD)</td>
<td>0.33±0.21</td>
<td>0.67±0.21</td>
<td>0.83±0.31</td>
<td>0.67±0.21</td>
<td>0.523</td>
</tr>
<tr>
<td>Vascular vacuolization and hypertrophy (VVH)</td>
<td>0.17±0.17*</td>
<td>0.67±0.21</td>
<td>1.17±0.31</td>
<td>0.50±0.22</td>
<td>0.045</td>
</tr>
<tr>
<td>Tubular cell degeneration and necrosis (THDN)</td>
<td>0.33±0.21</td>
<td>0.67±0.21</td>
<td>0.83±0.17</td>
<td>0.83±0.17</td>
<td>0.235</td>
</tr>
<tr>
<td>Bowman space dilation (BSD)</td>
<td>0.50±0.22</td>
<td>0.67±0.21</td>
<td>0.83±0.17</td>
<td>0.67±0.21</td>
<td>0.927</td>
</tr>
<tr>
<td>Tubular hyaline cylinder cell (THS)</td>
<td>0.50±0.22</td>
<td>0.67±0.21</td>
<td>0.83±0.17</td>
<td>0.67±0.21</td>
<td>0.724</td>
</tr>
<tr>
<td>Lymphocyte infiltration (LI)</td>
<td>0.17±0.17*</td>
<td>0.83±0.17</td>
<td>1.50±0.43</td>
<td>0.83±0.31</td>
<td>0.033</td>
</tr>
<tr>
<td>Tubular cell loss (THD)</td>
<td>0.33±0.21</td>
<td>0.67±0.21</td>
<td>0.83±0.31</td>
<td>0.83±0.31</td>
<td>0.508</td>
</tr>
</tbody>
</table>

* - Kruskal–Wallis test p < 0.05; **p < 0.05: Compared to Group S
Results

Malondialdehyde levels in Group S were significantly higher than those in all other groups \((p = 0.039, p = 0.021, p = 0.013\), respectively). MDA levels in Group FS and Group C were similar \((p = 0.624\). CAT enzyme activity in Group S was significantly higher than that in all other groups \((p = 0.003, p = 0.035, p = 0.002\), respectively) (Tab. 1).

The comparison of CAT enzyme activity between Group C and Group FS showed similar results \((p = 0.795\). PON enzyme activity in Group S was significantly lower than in groups C and FS \((p = 0.007, p = 0.039\), respectively). However, the comparison of PON levels between groups C and FS did not show any significant difference \((p = 0.447\) (Tab. 1).

The histopathological examination showed that vascular vacuolization and hypertrophy (VVH) and lymphocyte infiltration (LI) were significantly higher in Group S compared to Group C \((p = 0.006, p = 0.004\) (Tab. 2, Figs 1–4). The administration of Fullerol C60 before sevoﬂurane administration was not statistically significant (Tab. 2).

Discussion

The examination of the protective effect of fullerenols on sevoﬂurane-related renal injury showed that pretreatment with 100 mg/kg intraperitoneal fullerol effectively decreased renal injury in sevoﬂurane-administered rats. Sevoﬂurane, the potent volatile anesthetic undergoes degradation both in vivo and in vitro. The metabolism produces inorganic fluoride \((21)\) and the reaction with carbon dioxide absorbents produces the compound A \((34)\). Both degradation products can damage rat kidneys \((14)\). The concentration of compound A and the duration of exposure to compound A determine the extent of renal injury in rats \((35)\). The threshold for nephrotoxicity seems to be three hours of sevoﬂurane exposure. In the present study, the rats were administered with sevoﬂurane for 3 hours and we sought to determine whether fullerol was an effective agent against sevoﬂurane-related renal injury.

Srdjenovic et al \((36)\) demonstrated that fullerol given in a dose of 100 mg/kg could antagonize doxorubicin-induced toxicity in lungs, kidneys, and testes of rats. Based on their findings, our study was designed to test whether the fullerol in a dose of 100 mg/kg protects against kidney tissue damage related to sevoﬂurane inhalation. The results of this study showed that fullerol administered intraperitoneally 30 minutes before sevoﬂurane...
inhalation in rats reduced oxidative stress and partially corrected the damage caused by anesthesia in renal histopathology.

Malondialdehyde is one of the best-investigated products of lipid peroxidation. Lipid peroxidation products, including MDA, are produced from polyunsaturated fatty acids (PUFAs) by both chemical reactions and re-actions catalyzed by enzymes. (37). MDA is the prototype of the TBARS and it is the most frequently measured biomarker of oxidative stress, namely of lipid peroxidation. In many diseases, higher concentrations of MDA are measured in biological samples as compared to healthy individuals. Therefore, the elevated oxidative stress is generally regarded as a pathological condition. In the present study, the extent of damage in kidney was measured by MDA levels in rat renal tissues.

Catase is a type of conjugate that uses iron porphyrin as its prosthetic group and has a strong radical scavenging function that can protect the tissues from oxidative damage (38). With the action of CAT, H2O2 transforms into water and O2, thus preventing H2O2 from reacting with O2− and producing OH• in the presence of iron-chelating agents (39). When CAT inactivates H2O2, its consumption increases and thereby causes the deterioration of its activity. The results of this study indicated that the CAT content of renal tissue was significantly reduced following fullerenol treatment when compared with the group that received only sevoflurane.

Paraoxonases compose a family with three members (Paraoxonases 1, 2, and 3) that have various roles in multiple biochemical pathways including inflammation. Paraoxonase 1 (PON-1) is the most studied enzyme of the family. Therefore, in this study, PON-1 levels in the renal tissues were used to determine the protective effects of fullerenol. PON-1 plays a significant role in delaying/inhibiting the oxidation and in preventing the accumulation of lipid peroxides (40). The results of this study suggested that antioxidant activity of PON-1 was an important factor which provided protection from oxidative stress and lipid peroxidation against sevoflurane-related renal injury.

Conclusion

Our results confirm a satisfactory nephroprotective efficacy of fullerenol in the acute phase of sevoflurane-related renal toxicity and encourage further studies regarding its use as a potential nephroprotector.

References


Received September 21, 2019.
Accepted November 15, 2019.